AMENDMENTS TO THE SPECIFICATION

Please amend the specification as filed in the following manner:

Please replace the paragraphs at page 7, line 17 through page 10, line 19 with the following re-written paragraphs:

Figure 3 - Interaction with NEMO and functional kinase activity requires an IKKα-homologous region of the IKKβ COOH-terminus. **(A)** Truncation mutations of IKKβ sequentially omitting the extreme COOH-terminus (1-733), the serine-free region (1-707), the serine rich-domain (1-662) and the α_1-region (1-644) were expressed and labeled by *in vitro* translation and used for GST pull-down by GST-NEMO (Figure 1A). None of the mutants interacted with GST alone. **(B)** Sequence alignment of the extreme COOH-termini of IKKβ and IKKα. The α_2- and glutamate-rich regions are indicated and the six identical amino acids are shaded. **(C)** Wild-type IKKβ and the truncation mutants (1-733 and 1-744) were [^{35}S]-methionine-labeled (input) and used for *in vitro* pull down with either GST alone or GST-NEMO. **(D)** ~~HeLa cells were transfected for forty-eight hours with 1 μg/well of the indicated FLAG-tagged constructs followed by immunoprecipitation using anti-FLAG. The immunoprecipitates were incubated in kinase buffer containing [^{32}P] labeled γATP for fifteen minutes at 30°C then washed with lysis buffer containing 1% Triton-100. Resulting complexes were separated by SDS-PAGE (10%) and visualized by autoradiography (upper panel). The lower panel is an immunoblot from identical samples demonstrating equivalent amounts of transfected protein in each lane.~~ **~~(E)~~** HeLa cells were transfected for 48 hours with 1 μg/ml of the indicated constructs or empty vector (pcDNA-3) together with pBIIx-luciferase. NF-κB activity was determined by luciferase assay. ~~**(F)** HeLa cells transfected for forty-eight hours with FLAG-tagged versions of either IKKβ (wild-type) or IKKβ (1-733) were either untreated (-) or treated for seven minutes (+) with TNFα (10 ng/ml). Following lysis and immunoprecipitation using anti-FLAG, immune complex kinase assay (upper panels) was performed. Identical samples were immunoprecipitated and immunoblotted with anti-FLAG (lower panels).~~

Figure 4 - Association of NEMO with IKKβ and IKKα reveals the NEMO binding domain (NBD) to be six COOH-terminal amino acids. **(A)** COS cells transiently transfected with

vector alone, FLAG-tagged IKKα or IKKβ (1 μg/well) or xpress-tagged NEMO (1 μg/well) to a total DNA concentration of 2 μg/well as indicated. Following lysis, immunoprecipitations (IP) were performed using anti-FLAG (M2) and the contents of precipitates visualized by immunoblotting (IB) with either anti-FLAG (M2) or anti-xpress. A portion of pre-IP lysate was immunoblotted with anti-xpress to ensure equivalent levels of NEMO expression in transfected cells. **(B)** NEMO interacted equally well with both IKKβ and IKKα. **(C)** Wild-type IKKα and IKKα-(1-737) were expressed and labeled (input) and used for GST pull-down using GST or GST-NEMO. **(~~C~~ D)** Full length cDNA encoding human IKK$_i$ was obtained by RT-PCR from HeLa cell mRNA using the Expand™ Long Template PCR System (Boehringer Mannheim), the forward primer (5'-CTAGTCGAATTCACCATGCAGAGCACAGCCAATTAC) (SEQ ID NO: 22) and the reverse primer (3'-CTAGTCTCTAGATTAGACATCAGGAGGTGCTGG) (SEQ ID NO: 23) and cloned into the *EcoR*I and *Xba*I sites of pcDNA-3. GST pull-down analysis was performed using [^{35}S]-methionine-labeled IKKα, IKKβ and IKK$_i$. **(~~D~~ E)** A deletion mutant of IKKβ lacking the NBD (del.NBD) was [^{35}S]-methionine-labeled (input) and used for GST pull down analysis. **(~~E~~ F)** A Fauchere-Pliska hydrophobicity plot of the COOH-terminus (N721-S756) of human IKKβ was generated using MacVector™ (version 6.5.3) software. The NBD (L737-L742) is boxed. **(~~F~~ G)** COS cells were transfected for forty-eight hours with a total of 2 μg DNA/well of either vector alone, vector plus NEMO-FLAG or NEMO-FLAG plus xpress-tagged versions of IKKβ-(1-744) containing point mutations within the NBD as indicated. Following lysis and immunoprecipitation using anti-FLAG (M2), immunoblot analysis was performed with either anti-FLAG or anti-xpress. The level of expressed protein in pre-IP lysate was determined by immunoblotting with anti-xpress (lower panel). **(~~G~~ H)** HeLa cells were transiently transfected for forty-eight hours with the indicated constructs together with pBIIX-luciferase and NFκB activity in lysate was measured by luciferase assay.

Figure 5 - A cell-permeable peptide spanning the IKKβ NBD inhibits the IKKβ/NEMO interaction, TNFα-induced NF-κB activation and NF-κB-dependent gene expression. **(A)** Sequences of wild-type and mutant forms of IKKβ NBD peptide. **(B)** GST-pull-down analysis was performed using either GST-NEMO-*in vitro* translated IKKβ (upper panel) or GST-IKKβ-(644-756)-*in vitro* translated NEMO (lower panel). The assay was performed in the absence (no peptide) or presence of increasing concentrations (125, 250, 500 or 1000 μM) of either mutant

(MUT) or wild-type (WT) NBD peptide. **(~~B~~ C)** HeLa cells were incubated with either peptide (200 μM) for the times indicated. Following lysis, the IKK complex was immunoprecipitated using anti-NEMO and the resulting immunoblot probed with anti-IKKβ. **(~~C~~ D)** ~~HeLa cells were transfected for forty-eight hours with pBIIX-luciferase then incubated for two hours in the absence (control) or presence of mutant or wild-type NBD peptide (100 and 200 μM of each). Subsequently the cells were either treated with TNFα (10 ng/ml) as indicated (left panel) or left untreated (right panel) for a further four hours after which NF-κB activation was measured by luciferase assay.~~ Gel image showing anti-NEMO immunoprecipitation. **(E)** Gel image showing anti-FLAG immunoblot. **(~~D~~ F)** HeLa cells were incubated for three hours with increasing concentrations (50, 100 or 200 μM) of each peptide followed by treatment for fifteen minutes with TNFα (10 ng/ml) as indicated (+). Following lysis, nuclear extracts were made and 10 μg of protein from each sample was used for EMSA using a specific [^{32}P]-labeled κB-site probe. ~~**(E)** Primary HUVEC were pre-incubated for two hours with of wild-type (left) or mutant (right) NBD peptides (100 μM) then stimulated with TNFα (10 ng/ml) for a further six hours. Control cells received no peptide. Cells were stained with either anti-E-selectin (H4/18) or a non-binding control antibody (K16/16) and expression was measured by FACS (FACSort, Becton Dickinson). The profiles show E-selectin staining in the absence (shaded) and presence (solid line) of TNFα and control antibody staining under the same conditions (dashed line, no TNFα; dotted line, TNFα).~~ **(G)** Gel image showing anti-Phospho-C-Jun immunoblot and anti-β-Actin immunoblot. **(H)** HeLa cells were transfected for forty-eight hours with pBIIX-luciferase then incubated for two hours in the absence (control) or presence of mutant or wild-type NBD peptide (100 and 200 μM of each). Subsequently the cells were either treated with TNFα (10 ng/ml) as indicated (top panel) or left untreated (bottom panel) for a further four hours after which NF-κB activation was measured by luciferase assay.

Figure 6 - The wild-type NBD peptide inhibits NF-κB-induced gene expression and experimentally induced inflammation. **(A)** Primary HUVEC were pre-incubated for two hours with wild-type (middle) or mutant (bottom) NBD peptides (100 μM) then stimulated with TNFα (10 ng/ml) for a further six hours. Control cells received no peptide. Cells were stained with either anti-E-selectin (H4/18) or a non-binding control antibody (K16/16) and expression was measured by FACS (FACSort, Becton Dickinson). The profiles show E-selectin staining in the absence (shaded) and presence (solid line) of TNFα and control antibody staining under the

same conditions (dashed line, no TNFα; dotted line, TNFα). **(B)** % control release of NO_2- in various samples. **(C)** PMA-induced ear edema in mice topically treated with either vehicle (VEH), dexamethasone (DEX) or NBD peptides was induced and measured as described in Example 8. Data are presented as mean differences in ear thickness ± SD (* = $p < 0.05$ compared with both untreated control [-] and vehicle [VEH]). **(~~B~~ D)** The effects of the NBD peptide compared with the effect of dexamethasone (DEX) on Zymosan (ZYM)-induced peritonitis in mice were determined as described again in Example 8. Control mice were injected with phosphate-buffered saline (PBS).

Please replace the paragraph at page 41, lines 8-26 with the following re-written paragraphs:

HeLa cells were transfected for forty-eight hours with 1 μg/well of the indicated FLAG-tagged constructs followed by immunoprecipitation using anti-FLAG. The immunoprecipitates were incubated in kinase buffer containing [^{32}P]-labeled γATP for fifteen minutes at 30°C then washed with lysis buffer containing 1% Triton-100. Resulting complexes were separated by SDS-PAGE (10%) and visualized by autoradiography. An immunoblot from identical samples demonstrated equivalent amounts of transfected protein in each lane. HeLa cells transfected for forty-eight hours with FLAG-tagged versions of either IKKβ (wild-type) or IKKβ-(1-733) were also either untreated (-) or treated for seven minutes (+) with TNFα (10 ng/ml). Following lysis and immunoprecipitation using anti-FLAG, immune-complex kinase assay was performed. Identical samples were immunoprecipitated and immunoblotted with anti-FLAG.

IKKβ COOH-terminal truncation mutants were next used to test the effects of NEMO association on basal and induced activity of IKKβ. ~~Figure 3D shows that truncation~~ Truncation of IKKβ at V644, eliminating the serine-rich region (see Figure 3A), resulted in complete loss of basal auto-phosphorylation. In contrast, a mutant containing the serine-rich region (1-733), exhibited significantly higher levels of auto-phosphorylation than wild-type IKKβ ~~(Figure 3D)~~. Intriguingly, the level of auto-phosphorylation of IKKβ-(1-744) which contains the NEMO-binding α-region, was identical to that observed with the wild-type kinase. To test the effects that these mutations have on basal kinase activity, mutants were transiently transfected into HeLa cells and NF-κB activity determined by luciferase assay as described in Example 1. ~~The results in Figure 3D demonstrate that~~ IKKβ-(1-644) did not induce NF-κB activity whereas IKKβ-(1-733) caused increased activation compared with wild-type (Figure ~~3E~~ 3D).

Furthermore, NF-κB activity induced by IKKβ-(1-744) was identical to that induced by wild-type IKKβ. These results demonstrate that basal auto-phosphorylation and kinase activity of IKKβ is dependent on the ability of NEMO to associate with the kinase. One explanation for these observations may be that NEMO recruits a phosphatase to the IKK-complex that regulates basal IKKβ function by targeting the serine-rich region of the COOH-terminus. Inability to bind NEMO therefore prevents phosphatase recruitment and causes increased phosphorylation within this region.

Please replace the paragraph at page 41, line 27 through page 42, line 9 with the following re-written paragraph:

To directly test the effect that loss of the α-region has on the catalytic activity of IKKβ, an immune-complex kinase assay was performed on lysate from transfected HeLa cells ~~(Figure 3F)~~. For immune-complex kinases assays, precipitates were washed with TNT then with kinase buffer (20 mM HEPES pH 7.5, 20 mM $MgCl_2$, 1 mM EDTA, 2 mM NaF, 2 mM β-glycerophosphate, 1 mM DTT, 10 μM ATP). Precipitates were then incubated for fifteen minutes at 30°C in 20 μl of kinase buffer containing GST-IκBα-(1-90) and 10 μCi [^{32}P]-γ-labeled ATP (Amersham-Pharmacia). The substrate was precipitated using glutathione-agarose (Amersham-Pharmacia) and separated by SDS-PAGE (10%). Kinase activity was determined by autoradiography. Phosphorylated proteins associated with the kinase complex appeared on autoradiographs because the immuno-precipitated complex was not removed prior to GST-substrate precipitation.

Please replace the paragraph at page 42, lines 10-24 with the following re-written paragraph:

Activity of IKKβ (wild-type) was low in untreated cells but was markedly enhanced after treatment with TNFα. Consistent with the data presented in Figure ~~3E~~ 3D, basal activity of IKKβ-(1-733) was significantly higher than wild-type, however this activity was not further enhanced by treatment with TNFα ~~(Figure 3F)~~. Furthermore, basal and TNFα-induced catalytic activity of IKKβ-(1-744) was identical to the activity of IKKβ (WT). In addition to phosphorylated GST-IκBα, auto-phosphorylated IKKβ proteins were also detected ~~(Figure 3F, top bands)~~. Following TNFα treatment, IKKβ (WT) and IKKβ-(1-744) became rapidly

autophosphorylated whereas the already high basal phosphorylation of IKKβ-(1-733) was only slightly enhanced ~~(Figure 3F)~~. A previous study showed that auto-phosphorylation serves to down-regulate TNFα-induced IKKβ activity by causing conformational changes within the protein (Delhase *et al.*, (1999) Science 284, 309-313). Taken together, these findings (~~Figures 3D-F~~ Figure 3D) demonstrate that in the absence of NEMO, IKKβ becomes auto-phoshorylated, basally active and refractory to TNFα-induced signals indicating that NEMO plays a fundamental role in the down-regulation as well as activation of IKKβ activity.

Please replace the paragraph at page 42, line 25 through page 43, line 5 with the following re-written paragraph:

An additional band representing a phosphorylated protein appeared only in the samples from TNFα-induced IKKβ (WT) and IKKβ-(1-744) transfected cells ~~(Figure 3F)~~. The molecular weight of this protein (49 kDa) strongly suggests that it is endogenous NEMO associated with the precipitated complex. This is supported by the absence of the band in either precipitate (+/- TNFα) from IKKβ-(1-733) transfected cells. This protein has been identified as phosphorylated NEMO by dissociating the precipitated complex in SDS and re-immunoprecipitating [^{32}P]-labeled NEMO using specific anti-NEMO antibodies. Induced phosphorylation of NEMO may therefore represent a further level of regulation of the activity of the IKK complex.

Please replace the paragraph at page 43, lines 8-28 with the following re-written paragraph:

Since the α$_2$-region of IKKβ strongly resembles the COOH-terminus of IKKα (Figure 3B), the ability of IKKα to interact with NEMO was tested. Immunoprecipitations from lysate of COS cells transiently transfected with xpress-tagged NEMO together with FLAG-tagged versions of either IKKα or IKKβ were performed using anti-FLAG as described in Example 1. Figures 4A and 4B show ~~shows~~ that NEMO interacted equally well with both IKKβ and IKKα. It is possible that in this experiment the interaction with IKKα is not direct but due instead to the formation of a complex containing endogenous IKKβ, FLAG-IKKα and xpress-NEMO. A GST-pull-down assays was therefore performed using GST-NEMO and [^{35}S]-methionine-labeled versions of either wild-type IKKα or a truncated IKKα mutant lacking the eight COOH-

terminal amino acids (1-737: Figure ~~4B~~ 4C). In agreement with the findings presented above (Figure 4A), but in contrast to previous reports (Mercurio *et al.*, (1999) Mol. Cell. Biol. 19, 1526-1538; Yamaoka *et al.*, (1998) Cell 93, 1231-1240; Rothwarf *et al.*, (1998) Nature 395, 297-300), wild-type IKKα interacted with NEMO *in vitro* whereas the truncated mutant did not (Figure ~~4B~~ 4C). These results not only demonstrate that IKKα interacts with NEMO but also shows that it does so via the COOH-terminal region containing the six amino acids shared between IKKα and the α_2-region of IKKβ (Figure 3B). Gene-targeting studies have demonstrated profound differences in the activation of IKKα and IKKβ by TNFα (Woronicz et al., (1997) Science 278, 866-869; Zandi *et al.*, (1997) Cell 91, 243-252; Mercurio *et al.*, (1997) Science 278, 860-866; DiDonato *et al.*, (1997) Nature 388, 548-554; Régnier *et al.*, (1997) Cell 90, 373-383).

Please replace the paragraph at page 44, lines 9-21 with the following re-written paragraph:

Further evidence that this short COOH-terminal sequence constitutes the NEMO-interaction domain of the IKKs was obtained when we tested the ability of the recently described IKK-related kinase IKK$_i$ (Shimada *et al.*, (1999) Int. Immunol. 11, 1357-1362) to interact with NEMO. Sequence comparison with IKKα and IKKβ (Shimada *et al.*, (1999) Int. Immunol. 11, 1357-1362; Woronicz *et al.*, (1997) Science 278, 866-869; Zandi *et al.*, (1997) Cell 91, 243-52; Mercurio *et al.*, (1997) Science 278, 860-866; DiDonato *et al.*, (1997) Nature 388, 548-554; Régnier *et al.*, (1997) Cell 90, 373-383) reveals that IKK$_i$ does not contain the α_2-region in its COOH-terminus (Shimada *et al.*, (1999) Int. Immunol. 11, 1357-1362) and consistent with this being the NEMO binding domain we found that IKK$_i$ does not interact with GST-NEMO in pull down assays (Figure ~~4C~~ 4D). This finding indicates that NEMO is not required for the functional activity of IKK$_i$ and this is supported by the inability of IKK$_i$ to respond to signals induced by either TNFα or IL-1β (Shimada *et al.*, (1999) Int. Immunol. 11, 1357-1362).

Please replace the paragraph at page 44, line 24 through page 45, line 20 with the following re-written paragraph:

Having determined that the α-region of IKKβ, and the equivalent six amino acid sequence of IKKα are critical for interaction with NEMO [designated NEMO binding domain (NBD)], a deletion mutant in IKKβ lacking the six amino acids from L737 to L742 (del.NBD) was constructed. This deletion mutant did not associate with GST-NEMO (Figure ~~4D~~ 4E). Examination of predicted structural and biochemical features of the NBD in context with surrounding residues suggests that it constitutes an inflexible hydrophobic "pocket" within a hydrophilic region of the IKKβ COOH-terminus (Figure ~~4E~~ 4F). This suggests a model in which the NBD becomes buried within the first α-helical region of bound NEMO (Figure 2) preventing its exposure to an aqueous environment thereby maintaining a strong inter-molecular interaction. Whether the interaction is indeed a function of this hydrophobicity remains to be determined, however we found that substitution of either W739 or W741 with alanine prevented association of NEMO with IKKβ (Figure ~~4F~~ 4G). Therefore each of these hydrophobic tryptophan residues is critical for maintaining a functional NBD. In addition, mutation of D738 to alanine also prevented NEMO interaction indicating that a negatively charged residue at this position is required for NBD function. In contrast to these mutations, substitution of L737, S740 or L742 with alanine did not affect NEMO binding. To test the effects of these alanine substitutions on IKKβ function, HeLa cells were co-transfected with each of the point mutants together with pBIIX-luciferase reporter. Consistent with the observation that the basal activity of IKKβ is increased in the absence of associated NEMO, IKKβ-(1-733) (Figure ~~3E~~ 3D), mutants that did not bind NEMO (D738A, W739A and W741A) activated NF-κB to a greater extent than wild-type IKKβ or IKKβ-(1-744) (Figure ~~4G~~ 4H). In contrast, mutants containing substitutions that did not disrupt NEMO association (L737A, S740A and L742A) induced NF-κB to the same level as the controls. These results indicate that NEMO plays a critical role in the down-regulation of intrinsic IKKβ activity.

Please replace the paragraph at page 47, lines 1-8 with the following re-written paragraph:

The sequences of the two NBD peptides used in this study were [DRQIKIWFQNRRMKWKK]TALDWSWLQTE (wild-type) (SEQ ID NO: 18) and

[DRQIKIWFQNRRMKWKK]TALDASALQTE (mutant) (SEQ ID NO: 19); Figure 5A). The *antennapedia* homeodomain sequence (Derossi *et al.*, (1994) J. Biol. Chem. 269, 10444-10450; U.S. Patent No. 5,888,762; U.S. Patent No. 6,015,787; U.S. Patent No. 6,080,724) is bracketed and the positions of the W→A mutations are underlined. Both peptides were dissolved in DMSO to a stock concentration of 20 mM. For all experiments DMSO alone controls were no different from no peptide controls.

Please replace the paragraph at page 47, lines 9-19 with the following re-written paragraph:

The wild-type NBD peptide consisted of the region from T735 to E745 of IKKβ fused with a sequence derived from the third helix of the antennapedia homeodomain that has been shown to mediate membrane translocation (Derossi *et al.*, (1994) J. Biol. Chem. 269, 10444-10450). The mutant was identical except that the tryptophan residues (W739 and W741) in the NBD were mutated to alanine. Figure ~~5A~~ 5B shows that the NBD (WT) but not the mutant dose-dependently inhibited *in vitro* pull-down of [^{35}S]-labeled IKKβ by GST-NEMO and [^{35}S]-labeled NEMO by GST-IKKβ-(644-756). To test the ability of the NBD peptides to enter cells and inhibit the IKKβ-NEMO interaction, HeLa cells were incubated with the peptides for different time periods and immunoprecipitated the IKK complex using anti-NEMO. In agreement with the *in vitro* data (Figure ~~5A~~ 5B), wild-type but not mutant disrupted the formation of the endogenous IKK complex (Figure ~~5B~~ 5C).

Please replace the paragraph at page 47, line 22 through page 48, line 9 with the following re-written paragraph:

The effects of the NBD peptides on signal-induced activation of NF-κB were investigated next. Analysis using electrophoretic mobility shift assays (EMSA) also demonstrated that only the wild-type NBD peptide inhibited TNFα-induced activation and nuclear translocation of NF-κB (Figure 5F). Further, after ~~After~~ transfecting HeLa cells with the pBIIX-luciferase reporter, cells were preincubated with wild-type or mutant peptides, treated with TNFα and NF-κB activation measured by the luciferase reporter assay. As shown in Figure ~~5C~~ 5H (~~left~~ top panel), the wild-type NBD peptide inhibited TNFα-induced NF-κB activation whereas the mutant had no effect. Interestingly, the basal NF-κB activity was enhanced by treatment with the wild-type

peptide (Figure ~~5C~~ 5H; ~~right~~ bottom panel), a finding which concurs with results from previous mutational analysis (Figures ~~3E-F~~ 3D and ~~4G~~ 4H). This indicates that removal of NEMO increases the basal, intrinsic activity of IKK, while abolishing its responsiveness to TNFα. ~~Further analysis using electrophoretic mobility shift assays (EMSA) also demonstrated that only the wild-type NBD peptide inhibited TNFα-induced activation and nuclear translocation of NF-κB (Figure 5D).~~ Taken together these results demonstrate that delivery of an intact NBD peptide into cells disrupts the IKKβ-NEMO interaction and prevents pro-inflammatory signals from activating NF-κB. In contrast, transduction with a peptide containing mutations at the tryptophan residues that are critical for maintaining the NEMO interaction has no effect.

Please replace the paragraph at page 48, lines 12-28 with the following re-written paragraph:

Many proteins involved in the initiation and maintenance of inflammatory responses require NF-κB activation for induced expression of their genes (Ghosh *et al.*, (1998) Annu. Rev. Immunol. 16, 225-260; May & Ghosh, (1998) Immunol. Today 19, 80-88). One such protein, E-selectin, is a leukocyte adhesion molecule expressed on the luminal surface of vascular endothelial cells after activation by pro-inflammatory stimuli such as IL-1 or TNFα (Pober *et al.*, (1986) J. Immunol. 436, 1680-1687; Bevilacqua *et al.*, (1987) Proc. Natl. Acad. Sci. USA 84, 9238-9242; Collins *et al.*, (1995) FASEB J. 9, 899-909). Expression of E-selectin and other NF-κB-dependent adhesion molecules is crucial for the arrest and recruitment of leukocytes into sites of acute and chronic inflammation. To assess the anti-inflammatory potential of the NBD peptide, primary human umbilical vein endothelial cells (HUVEC) were pretreated with the wild-type and mutant peptides and E-selectin expression induced with TNFα. Consistent with the effects on basal NF-κB activation (Figure ~~5C~~ 5H), the wild-type NBD peptide induced low level expression of E-selectin (Figure ~~5E~~ 6A). However, after TNFα-treatment the wild-type but not mutant significantly reduced expression of E-selectin (Figure ~~5E~~ 6A). Inhibition by wild-type NBD peptide reduced expression to the level induced by the peptide in the absence of TNFα.

Please replace the paragraph at page 50, lines 6-10 with the following re-written paragraph:

Figure ~~6A~~ 6C shows that the wild type peptide significantly reduced (77 ± 3% inhibition; $p < 0.05$) PMA-induced ear thickening to the level observed with dexamethosone (82 ± 9% inhibition; $p < 0.05$). In contrast, the effect observed with an equivalent dose of mutant was insignificant ($p = 0.09$). Neither peptide had an effect when administered in the absence of PMA (not shown).

Please replace the paragraph at page 50, lines 21-25 with the following re-written paragraph:

As shown in Figure ~~6B~~ 6D zymosan injection caused an accumulation of inflammatory exudate fluids and migration of polymorphonuclear cells (PMN) into the peritoneum of these animals. Treatment of mice with wild type NBD peptide or dexamethasone significantly reduced exudate formation and PMN accumulation whereas the mutant had no effect.

Please replace the paragraph at page 50, line 26 through page 51, line 10 with the following re-written paragraph:

Various *in vivo* studies have demonstrated a role for NO in exudate formation and leukocyte migration into inflammatory sites (Ialenti *et al.*, (1992) Eur. J. Pharmacol. 211, 177-182; Ialenti *et al.*, (1993) Br. J. Pharmacol. 110, 701-706; Iuvone *et al.*, (1998) Br. J. Pharmacol. 123, 1325-1330). Therefore the effects of the NBD peptides on NOX accumulation in the peritoneal exudates of zymosan-treated mice ~~was~~ were investigated. Figure ~~6B~~ 6D (lower panel) shows that dexamethasone and wild-type peptide reduced NOX by 86 ± 7% and 66 ± 4% respectively whereas the mutant had no effect. These results are consistent with previous studies demonstrating that reduction of exudate formation and cell accumulation closely correlate with inhibition of NF-κB activation and reduction of NO formation (D'Acquisto *et al.*, (1999) Eur. J. Pharmacol. 369, 223-236; D'Acquisto *et al.*, (1999) Naunyn-Schmeideberg's Arch. Pharmacol. 360, 670-675). Therefore the wild-type NBD peptide is an effective inhibitor of inflammation in experimental animal models.